

I / We Claims

1. An oligonucleotide primer pair having SEQ ID NO: 3 and SEQ ID NO: 4 for amplification of Early Secretory Antigenic Target (*esat*)-6-gene of *Mycobacterium* species.
- 5 2. A method for detecting *M. tuberculosis* in a sample based on the amplification of *esat*-6 gene, the said method comprising the steps of :
 - a) isolating DNA template from the sample,
 - d) amplifying the DNA template by adding a reaction buffer, oligonucleotide primer pair having SEQ ID NO: 3 and SEQ ID NO: 4, and heat stable DNA
10 polymerase to obtain an amplified DNA product, and
 - e) subjecting the amplified DNA product of step (b) to separation, and staining to detect the presence of amplified DNA product wherein the presence of amplified DNA product is indicative of *Mycobacterium tuberculosis* in the sample.
- 15 3. A method according to claim 2, wherein the sample is either clinical sample or culture sample.
4. A method according to claim 3, wherein the clinical samples is selected from a group of sputum, bronchoalveolar, lavage fluid, pleural fluid, ascetic/peritoneal fluid, cerebrospinal fluid (CSF), pus fecal matter, urine, amniotic fluid, menstrual
20 blood, peripheral blood or other body fluids, lymph node, pus or other aspirate, and tissue biopsies.
5. A method as claimed in 2 wherein in step (b) the amplification is by polymerase chain reaction.
6. A method as claimed in 2 wherein the amplification consists of 25-35 cycles of
25 amplification.
7. A method according to claim 2, wherein in step (b) the heat stable DNA polymerase is *Taq polymerase*.
8. A method as claimed in 2 wherein in step (c) the separation is done preferably by gel electrophoresis.
- 30 9. A method as claimed in 2 wherein in step (c) the staining is by ethidium bromide.

10. A method as claimed in 2 wherein in step (c) the amplified DNA product is 320 base pair in length.
11. A diagnostic kit for detection of *Mycobacterium tuberculosis*, from other species of *Mycobacteria* comprising of oligonucleotides primers having SEQ ID NO: 3 and
5 SEQ ID NO: 4, all four deoxyridonucleotide triphosphate (dNTPs), reaction buffer, *Taq polymerase*, DNA marker, positive and negative control and instruction manual.
12. A method for detecting *M. tuberculosis* based on amplification wherein, the said method comprising the steps of:
- 10 i. amplifying the 16s rRNA region from the isolated DNA template using the primer pair having SEQ ID NO: 1 and SEQ ID NO: 2 to obtain first amplified product using conventional method,
- ii. detecting the amplified product of step (a) wherein the presence of 1030 base pair amplified DNA product is indicative of positive sample for the presence of *Mycobacterium* species,
- 15 iii. employing the DNA from the positive samples identified from step (b) for further detection of *M. tuberculosis* based on the amplification of *esat-6* gene,
- iv. amplifying the *esat-6* gene using the primer pair having SEQ ID NO: 3 and SEQ ID NO: 4 to obtain second amplified product using method as claimed
20 in claim 2, and
- v. detecting the amplified product of step (d) wherein the presence of 320 base pair is indicative of *Mycobacterium tuberculosis* in the sample and absence is indicative of other *Mycobacterium* species.
13. A method according to claim 12, wherein the DNA template is obtained either from
25 clinical sample or from culture sample.
14. A method according to claim 13, wherein the clinical sample is selected from a group of sputum, bronchoalveolar, lavage fluid, pleural fluid, ascetic/peritoneal fluid, cerebrospinal fluid (CSF), pus fecal matter, urine, amniotic fluid, menstrual blood, peripheral blood or other body fluids, lymph node, pus or other aspirate, and
30 tissue biopsies.

15. A method as claimed in 12 wherein the amplification is by polymerase chain reaction.
16. A method according to claim 15 wherein the amplification is by heat stable DNA polymerase such as *Taq polymerase*.
- 5 17. A method as claimed in 12 wherein in step (i) the amplification consists of 30-40 cycles of amplification.
18. A method as claimed in 12 wherein in step (iii) the amplification consists of 25-35 cycles of amplification